Diet and serum lipid concentrations: where are we?1,2

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INTRODUCTION

Hegsted et al (1) evaluated the data published through 1991 on the effects of the fatty acid classes—saturated, monounsaturated, and polyunsaturated—and dietary cholesterol on serum cholesterol and lipoprotein concentrations from studies that used human subjects. Only studies in which diets composed of ordinary foods were prepared and given to the subjects and that provided data on all four variables—the three classes of fatty acids and dietary cholesterol—were considered. Studies designed to evaluate the effects of specific saturated fatty acids, n-3 and trans fatty acids, isomerized fats, fish oils, and weight-reducing diets were eliminated. There were 248 observations that met these criteria. Field trials, in which only limited dietary control or information can be provided, were also analyzed but for obvious reasons these data must be considered to be of less quantitative value.

This analysis (1) supported the general conclusions of Keys et al in 1957 (2) and Hegsted et al in 1965 (3) that saturated fatty acids elevate serum cholesterol and are the primary determinant of serum cholesterol concentrations, that polyunsaturated fatty acids (primarily linoleic acid) actively lower serum cholesterol, and that monounsaturated fatty acids (primarily oleic acid) have little or no effect. Dietary cholesterol may also elevate serum cholesterol but it has a lesser effect than does fat. However, the quantitative estimates of the effects of each component differed somewhat from the earlier estimates.

The database that included data on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) was much more limited (155 observations), but indicated, as expected, that the dietary effects on LDL approximate those of total serum cholesterol. The data on HDL did not allow the development of a meaningful predictive equation (R^2 = 0.37) but may have been of interest in that the regression coefficients for all three classes of fatty acids were positive, suggesting that they all tended to elevate HDL concentrations, with saturated fatty acids being the most effective and polyunsaturated fatty acids the least.

Numerous papers have appeared since that time aimed at evaluating the same issues. The results have been highly variable. This leads us to ask what has been achieved by these numerous and expensive studies, where this field is going, and especially, why there appears to be so much disagreement in studies of this kind? We believe that many reports include inadequate data and reach inappropriate conclusions, but that such studies may not be identified easily. There are, however, obvious sources of misinformation and poor-quality data that are often ignored.

DIETARY CONTROL

The first source of misinformation and data of poor quality is the lack of adequate dietary control. Quantitative nutrition studies require quantitative information about the foods eaten. The only way that this can be assured is by institutionalizing the subjects, but this is now practically impossible because of cost and the inability to keep subjects in metabolic wards for extended periods. Practically all authors have been forced to use free-living subjects. In the "better studies" all foods are prepared for the subjects but many meals are not eaten under supervision. Most authors have made some attempt to estimate compliance—with food records, for example—and some have measured serum fatty acids. The latter can show that the diet did change but does not provide a measure of the degree of compliance. It is certain that compliance is not absolute and no adequate methods exist to determine how good or bad the estimates of compliance really are.

It is now clear from measures of energy expenditure (4–7) that many individuals do not accurately report their energy consumption and that most underestimate consumption. This must mean that estimates of the consumption of most other nutrients is also poor. Fat consumption is particularly difficult to estimate because of the variability in the fat content of similar foods and because the fat content of a food varies with the method of preparation. What is worse is that experimental subjects, who have been well-instructed about how they should perform and drilled on the importance of following instructions, may be less reliable than untrained subjects (8). People who have committed themselves, who receive free meals, and who may be paid are likely to minimize failures to follow instructions that might eliminate them from the study.

Some studies report the elimination of a few subjects because of noncompliance, but it is unclear how this noncompliance was detected. Clearly, investigators are dependent on the veracity of the subjects, some noncompliance always occurs, and any degree of noncompliance diminishes the differences in serum lipid response that would occur had compliance been absolute. All such errors diminish the likelihood that significant differences will be found. The degree of compliance or noncompliance can never be determined from published data.

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DIET COMPOSITION

Similar foods may vary substantially in composition, and analytic values may or may not agree with calculated values (9, 10). Reported studies vary greatly in the care taken to define the experimental diets and ensure their constancy. In many studies, the baseline or usual diet is used to evaluate the response. The composition of the usual diet is based on food intake records of some kind and must be calculated from food-composition tables. The reliability of such data is not known and, even if the average diet is correctly defined, this does not represent the diet of each subject.

There have been numerous reports of field trials using supplements with instructions on how the diet should be modified. Such studies depend entirely on reported food intakes and calculated values and, obviously, the average diet does not represent the diet of each individual. These kinds of trials may be of value in evaluating what can be accomplished by the methods used but they are not quantitative tests of the effects of diet and should be clearly distinguished from better-controlled trials. They have, however, been interpreted as quantitative studies, as in the meta-analysis published recently by Gardner and Kraemer (11).

CHARACTERISTICS OF SUBJECTS

There are numerous studies that show, as did Keys et al in 1959 (12), that the response to dietary change may be markedly affected by the baseline cholesterol concentration of the subjects studied. Individuals with relatively low serum cholesterol concentrations have only limited capacity to respond to cholesterol-lowering diets. Yet, unfortunately, many recent data have been collected using students and young adults with low cholesterol concentrations as subjects. We believe that the primary interest should be to define diets that lower cholesterol concentrations in individuals at risk or diets that prevent the development of lipid concentrations that put them at risk. Such diets cannot be defined with data collected from subjects with a limited capacity to respond.

It is also clear that apparently similar subjects may respond differently to the same dietary treatment, i.e., hypo- and hyper-responders. The fact that a large proportion of Americans have cholesterol concentrations that put them at risk shows that most Americans respond unfavorably to the American diet, but small studies may contain an unusual proportion of hypo- or hyperresponders.

A large SD in baseline cholesterol, indicating substantial differences in the response to the baseline diets, is likely to yield a large variation in response to the experimental diet. Such variation makes it more difficult to detect significant differences in response. The more homogeneous the group studied, the more likely it is that intervention will be significant. The use of a very heterogeneous group limits the reliability of any trial.

DEFINITION OF LIPID CONCENTRATIONS

It is well known that serum cholesterol concentrations in the same individual vary greatly from one blood sampling to the next (13–16). The SD of cholesterol concentrations within individuals is usually of the order of 10% of the mean, but is often greater for some individuals so that even three or four samples may not define the concentration in some individuals very well. Studies vary greatly but many are based on too few blood samples. This is particularly worrisome when few subjects are studied. A review of cholesterol variation among the subjects in the 10 American Lipid Research Clinics suggested that total and LDL-cholesterol concentrations may vary inversely with hours of daylight (17).

Although it is agreed that LDL is the atherogenic lipoprotein, there is no evidence that measurement of LDL provides a better prediction of risk than does total serum cholesterol. The measurement of LDL is inherently less accurate than that of total cholesterol. HDL concentrations are meaningful in epidemiologic data, but it is not entirely clear how to interpret diet-induced changes in HDL concentrations. The metabolism of HDL may be modified when lowered by dietary means (18) and low-fat diets, which clearly protect against coronary heart disease, are associated with low HDL concentrations (19). Hence, it is uncertain that an induced reduction of HDL implies the same increased risk as it does in population studies.

OTHER FOOD COMPONENTS

It is now abundantly clear that there are many compounds in foods and in fats and oils, other than the fatty acids and cholesterol, that affect serum lipid concentrations. These include plant sterols, dietary fiber, sources of protein, coffee, tocotrienols, and probably other materials not yet identified. Diets or oils with similar fatty acid contents differ in their content of such components. The relative amounts of these other materials are never defined, but it is reasonable to believe that such materials may be the cause of some apparently aberrant results. Olive oil, the oil used in many trials that attempt to assess the effects of the monounsaturated fatty acids and the major fat in the Mediterranean diet, may be particularly variable because of the many grades and methods of preparation of the oil. As yet, we have no information on the quantitative importance of such materials relative to the fatty acids nor is it clear whether these materials act independently or affect the response to the various fatty acids. It would seem certain that not all of the effects observed when dietary fats are varied are due to differences in fatty acids. It is dangerous to generalize from small studies that compare only a few fats or oils.

There are also data indicating that the serum lipid response to specific fatty acids may be dependent on the cholesterol content of the diet (20, 21). Presumably, this explains the atypical response to cholesterol-free formula diets that lower serum cholesterol even when they contain saturated fats and why they consistently fail to distinguish between the effects of mono- and polyunsaturated fatty acids (22). Prediction equations that include data derived with formula diets (23) result in small regression coefficients for the fatty acids, as would be expected.

MOLECULAR CONFIGURATION OF TRIACYLGLYCEROLS

McGandy et al (24) trans-esterified olive oil and safflower oil to produce products containing approximately equal pro-
portions of lauric, myristic, palmitic, and stearic acids to evaluate the effects of the major saturated fatty acids. Contrary to expectation and data obtained with some natural fats, such as cocoa butter, these preparations had approximately equal cholesterol-raising activities. In an earlier study, pure triacylglycerols were trans-esterified with corn oil to yield fats rich in lauric, myristic, palmitic, or stearic acids; there were no differences in their effects on experimentally induced atherosclerosis in rabbits (25).

In most fats, the polyunsaturated fatty acids are in the β position in the triacylglycerol molecule; this position is largely (75–80%) conserved during the process of digestion. Most saturated fatty acids are in the 1 and 3 positions and are freed during digestion. Either or both fractions may affect the cholesterol-raising or -lowering activity. The effect of some natural oils may reflect their molecular structure. Tallow and lard both contain ~24% palmitic acid. In lard, almost all the palmitic acid is in the sn-2 position, whereas in tallow it is primarily at positions 1 and 3. Lard is significantly more atherogenic for rabbits than tallow but on intersterification the atherogenicity of the two fats becomes virtually identical and significantly less atherogenic than unaltered lard (26). Synthetic triacylglycerols offer obvious opportunities for modifying fats either favorably or unfavorably but the results obtained with such fats should not be extrapolated to ordinary fats until more is known (27).

SAMPLE SIZE

It is clear that larger sample sizes have more statistical power to define differences. It is invariably true, however, that the degree of dietary control is increasingly compromised as sample size increases. This severely limits the options available in dietary studies. Very large trials generally result in minimal changes in serum lipids, inconsistent with reported food intakes (28). Unfortunately, such results are often interpreted as evidence of the lack of effect of dietary modification (29). Large field trials designed to evaluate the effects of diet on disease require measures of individual compliance because those who develop disease may be those who are not in compliance. Because reliable measures of compliance are not available, such trials are likely to severely underestimate the true potential of dietary modifications.

STATISTICAL TREATMENT

Standard statistical analysis often confuses the issue. If the differences in treatment are not significant, ie, \( P > 0.05 \), the common conclusion is that no differences exist or that the treatments produce the same result. This is not generally true. Nonsignificance simply indicates that the data do not allow the conclusion that the results do differ. In many studies the SEs are large and very large mean differences are required to produce significance. Many do not have the capacity to evaluate the hypothesis being tested. The differences observed may be important even if they do not fit the statistical criteria. As McClosky concludes in his article, The Insignificance of Statistical Significance (30), "Scientists care about whether a result is statistically significant, but they should care much more about whether it is meaningful."

As one example out of many, Wardlaw et al (31) reported that a diet high in monounsaturated fat and one high in polyunsaturated fat were equally efficacious in lowering LDL cholesterol. The former produced a decrease of 12% and the latter 20%—which were not significantly different—although a difference of 8% would be considered substantial in a public health context. The SEM was 2% in both instances. Thus, the true value of the monounsaturated fat group might be as low as 8% (12 ± 2 SEMs) and the polyunsaturated fat value might be as high as 24% (20 ± 2 SEMs)—a total difference of 16% in favor of the diet high in polyunsaturated fat. Emphasizing confidence limits, which is standard practice in epidemiologic publication, rather than statistical significance would at least allow the reader to note how well the mean values have been defined. Also note that in the multitude of comparisons now reported, there is the likelihood that 1 in 20 will show significant differences by chance alone.

The great majority of the studies done during the past 10–15 y have reevaluated the familiar issues, namely the effects of dietary cholesterol or the effects of the three fatty acid classes, especially the effect of the monounsaturated fatty acids relative to the polyunsaturated fatty acids. The variable results have produced more confusion than clarification, which is due, in part, to flaws in design or execution of varying degrees of seriousness. Investigators and editors should pay attention to the issues that we have discussed—the reliability of the dietary data and how they were obtained, the variance in the data, whether the study has the capacity to distinguish between the groups being evaluated, and whether the conclusions, whatever they may be, are fully justified. The "prevention paradox" should be recalled, ie, that modest differences that are of little or no significance in treating individuals may be very important in public health applications.

We do not imply that nothing has been learned in this field in recent years but we do believe that the yield has been small. In particular, quantitative estimates of changes in serum lipids induced by dietary changes are likely to be underestimated and this should be borne in mind.

Repetition of studies to confirm prior findings is a necessity. It seems useless and confusing, however, to simply repeat the same studies over and over again unless there are improvements in technique or quality. Neither appears to be true of recent studies. It is time to try to define those issues now worthy of further investigation, the characteristics studies must have if they are to be successful, and whether we have the technical capacity to develop definitive answers.

REFERENCES